The first recorded outbreak of cryptosporidiosis due to Cryptosporidium cuniculus (formerly rabbit genotype), following a water quality incident

Richard L. Puleston, Cathy M. Mallaghan, Deborah E. Modha, Paul R. Hunter, Jonathan S. Nguyen-Van-Tam, Christopher M. Regan, Gordon L. Nichols and Rachel M. Chalmers

ABSTRACT

We report the first identified outbreak of cryptosporidiosis with Cryptosporidium cuniculus following a water quality incident in Northamptonshire, UK. A standardised, enhanced Cryptosporidium exposure questionnaire was administered to all cases of cryptosporidiosis after the incident. Stool samples, water testing, microscopy slides and rabbit gut contents positive for Cryptosporidium were typed at the Cryptosporidium Reference Unit, Singleton Hospital, Swansea. Twenty-three people were microbiologically linked to the incident although other evidence suggests an excess of 422 cases of cryptosporidiosis above baseline. Most were adult females; unusually for cryptosporidiosis there were no affected children identified under the age of 5 years. Water consumption was possibly higher than in national drinking water consumption patterns. Diarrhoea duration was negatively correlated to distance from the water treatment works where the contamination occurred. Oocyst counts were highest in water storage facilities. This outbreak is the first caused by C. cuniculus infection to have been noted and it has conclusively demonstrated that this species can be a human pathogen. Although symptomatically similar to cryptosporidiosis from C. parvum or C. hominis, this outbreak has revealed some differences, in particular no children under 5 were identified and females were over-represented. These dissimilarities are unexplained although we postulate possible explanations.

Key words | Cryptosporidium, outbreak, rabbit, water quality

INTRODUCTION

We report an outbreak of Cryptosporidium cuniculus following a water quality incident in Northamptonshire, UK.

Cryptosporidiosis is a faeco-ora lly transmitted diarrhoeal illness, caused by species of the parasitic protozoan genus Cryptosporidium. There are at least 26 recognised Cryptosporidium species (Chalmers & Katzer 2013), but C. hominis and C. parvum predominate in causing human disease (Davies & Chalmers 2009). Oocysts may be found in any faecally contaminated water and resist chlorine disinfection as is commonly used in producing potable water (Davies & Chalmers 2009; Medema et al. 2009; Yoder & Beach 2010).
In industrialised countries, symptoms are most commonly reported in young children (aged 1 to 5 years) (O’Donoghue 1995; Chalmers et al. 2009a). Symptoms, which can be relapsing, persist for up to three weeks, and longer in the immunocompromised (Hunter & Nichols 2005; Cacciò et al. 2005; Abubakar et al. 2007; Davies & Chalmers 2009). Non- C. parvum and non- C. hominis species and genotypes cause disease mainly in the immunocompromised (Elwin et al. 2011).

Cryptosporidiosis outbreaks have been associated with recreational and drinking water use, farm visiting, childcare facilities and contaminated foods and beverages (Baldursson & Karanis, 2011; Robertson & Chalmers 2013).

We focus on the epidemiological characteristics of cases arising from this outbreak and discuss the differences from previous incidents.

**METHODS**

On 23 June 2008, a small amount of Cryptosporidium sp. oocyst contamination (0.0005 oocysts/L) was noted, by continuous (but not real-time) inline water filtration cartridge monitoring (commenced 19 June), of the drinking water supply to approximately 258,000 people in central and western Northamptonshire (UK), provided from a surface water reservoir and treatment works based in the county (Drinking Water Inspectorate 2009). Repeat sampling taken up to the evening of 24 June showed a further rise. Local public health authorities were notified and a water supply emergency declared at 06.00 hours on 25 June. Control measures were instituted, including ‘boil water’ messages to users in the locality (Drinking Water Inspectorate 2009). Repeat sampling taken up to the evening of 24 June showed a further rise. Local public health authorities were notified and a water supply emergency declared at 06.00 hours on 25 June.

Control measures were instituted, including ‘boil water’ messages to users in the locality (Drinking Water Inspectorate 2009). To assess the extent of contamination, further water sampling was undertaken (continuous filtration and grab sampling) at strategic points in the distribution network, including from end user sites. A search for biosecurity failures was instigated and remediation initiated. Network flushing and storage reservoir decontamination was undertaken.

Local health professionals were alerted and requested to submit stool samples for laboratory analysis from suspected cases of cryptosporidiosis and to notify the local public health authorities of such cases.

**Case definition**

After the incident it was unclear if any cases of cryptosporidiosis would occur. Existing surveillance systems were used to identify possible cases using the following case definition: ‘cases of diarrhoea/gastrointestinal illness occurring in individuals residing in the affected area with microbiologically confirmed Cryptosporidium sp. (later tightened to C. cuniculus), having consumed mains water between 19 June and 06.00 hours on 25 June (when the boil water notice was issued)’.

A standardised, enhanced Cryptosporidium exposure questionnaire was administered by telephone, post or in person to all cryptosporidiosis cases notified to the Health Protection Agency (HPA) in the weeks following the incident. Details of symptoms, water consumption, co-morbidities and medication history were obtained. Cryptosporidium sp. isolates were typed at the national Cryptosporidium Reference Unit, Singleton Hospital, Swansea (Chalmers et al. 2009b).

The distance of each case’s home from the water treatment works was estimated using Microsoft Corp., MapPoint© (direct and by road (the latter chosen as water pipes, in part, follow road routes)).

**Statistical analysis**

All statistical analyses were undertaken in Stata (StataCorp Inc., version 10). Case characteristics were summarised. Interquartile ranges are reported for medians. The significance of the difference in means and proportions was calculated with two group mean and proportion tests respectively. Parametric (linear) and non-parametric (Spearman’s) regression analyses to assess the association between the date of onset of illness and volume of water drunk/distance from the water treatment works were estimated; p-values of ≤0.05 were considered statistically significant.

**RESULTS**

Between 09.29 hours on 19 June and 11.50 hours on 23 June, six oocysts in 11,848 L of treated water (0.0005
oocysts/L) were noted in a continuous filter cartridge sample, where normally none would be detected. Sampling repeated between 11.50 hours on 23 June and 20.00 hours on 24 June showed a count of 418 oocysts in 5,064 L water (0.08 oocysts/L). Over the same period, no oocysts were identified in the raw water; an overwhelming of the treatment capacity of the plant was therefore discounted. Source identification centred on a biosecurity breach from within the treatment works. During the incident the maximum count of oocysts noted was 1.7 oocysts/L (10 L grab sample) on 26 June at a clean water storage reservoir site distal to the treatment works. Sporadic oocysts were found up until 22 July from storage sites, although counts were below 0.01/L by 2 July. Oocyst counts from end-user customer taps, peaked at 0.19 oocysts/L (259 oocysts in 1,391 L) at one address on 25 June and 0.007 oocysts/L (9 in 1,166 L) at another on the same date and over a similar time frame – a 27-fold difference. Counts at customer taps dropped to below 0.01 oocysts/L by 29 June, but sporadic oocysts were found until 3 August. End-user monitoring continued until 5 August.

Investigations discovered (evening of 27 June) a fresh wild rabbit carcass (*Oryctolagus cuniculus*) immediately below the inlet pipe to a backwash granulated activated carbon tank. It was assumed that the oocysts had been released into the disinfection contact tank from the carcass. Defects in two vent covers and a granulated activated carbon tank access point had allowed the rabbit to enter the treated water (Northampton Borough 2009; Drinking Water Inspectorate 2009). The remaining gut contents contained *C. cuniculus* gp60 gene subtype VaA18 oocysts (Chalmers et al. 2009).

Up to the week ending 6 August, 32 microbiologically confirmed cases of cryptosporidiosis were notified. Twenty-three had *C. cuniculus* gp60 gene subtype VaA18 and were therefore linked to the incident epidemiologically and microbiologically (Chalmers et al. 2009b). Over the same period in the previous year, there were only four (unrelated) cases of cryptosporidiosis notified to the HPA from the same geographic area as this incident. Few data were available from one (non-responder). Although oocysts detected at end-user sites were not submitted for typing, seven water samples taken from other points in the network had *C. cuniculus* gp60 gene subtype VaA18 contamination, further strengthening the conclusion that the drowned rabbit was the source of the outbreak (Northampton Borough 2009).

There were seven male and 16 female cases (30% male); this difference approached but was not statistically significantly different (*p* = 0.061). One of the cases and possibly a second may have resulted from secondary infection. The mean age of cases was 32 (95% CI 26.6–37.4) years, (males 33 years, females 32 years, *p* = 0.90).

All presented with diarrhoea. The first developed symptoms on 24 June and the last on 14 July. The mean date of diarrhoea onset was 2 July (1 July males, 2 July females, *p* = 0.50).

Monitoring data indicated that the first possible date of contamination was 19 June and the last 23/24 June. However, the oocysts count per litre increased 165-fold from the sample completed on 23 June and the one commenced on 23 June and completed on 24 June and then reduced rapidly. Therefore, 23 June was assumed to be the most likely date of contamination.

Using 23 June as the exposure date, the incubation period for *C. cuniculus* ranged between 1 and 21 days, mean 9.2 (95% CI 7.4–11.0) days; males 8.3 (6.3–10.2) days and females 9.6 (7.1–12.1) days, *p* = 0.50. The median incubation period was 8 (interquartile range (IQR) 8–10) days; males 8 (IQR 7–10) days and females 8.5 (IQR 8–9.5) days. The mode was 8 days for both sexes.

The epidemic curve was similar for men and women (Figure 1); however, there were two late presenting females who did not share an address with any of the earlier cases, although one shared an address with a symptomatic individual from whom microbiological confirmation was not obtained. The median duration of diarrhoea was 13 (IQR 6–19) days, males 5.5 (IQR 5–15) days and females 14 (IQR 9–20) days. The data distribution and number of data points preclude reporting of the modal duration of diarrhoea. Other epidemiological cases characteristics, including other potential risk factors, are shown in Table 1.

### Water consumption

The median self-reported total daily mains water consumption was 2.3 L (IQR 1.6–3.3; mean 2.4 L); males 2.8 L (IQR 2.3–3.1; mean 2.7 L) and females 1.9 L (1.3–3.5; mean
2.3 L). The median unboiled water consumption was 1.8 L (IQR 1.0–2.4; mean 1.8 L); for males 2.0 L (IQR 1.8–2.4; mean 2.1 L) and females 1.6 L (IQR 0.75–2.7; mean 1.7 L). Median boiled water consumption was 0.6 L (IQR 0.3–1.0; mean 0.6 L), males 0.6 L (IQR 0.25–0.9; mean 0.6 L) and females 0.6 L (IQR 0.3–1.0; mean 0.6 L).

The volume of mains water consumption (total, unboiled or boiled) did not correlate to the incubation period or date of onset of diarrhoea. There was little correlation between diarrhoea duration and total water consumption (Spearman’s rho –0.02, p = 0.99) or unboiled or boiled tap water (Spearman’s rho 0.07, p = 0.77 and –0.28, p = 0.27 respectively).

**Distance from water treatment works**

The median distance of cases from the water treatment works was 6.24 (IQR 3.9–7.9) km direct and 9.8 (IQR 5.2–12.1) km by road. Neither correlated to the incubation period; however, the duration of diarrhoea was negatively correlated to the direct distance from the water treatment works (Spearman’s rho –0.4847, p = 0.0260).

**DISCUSSION**

**Cryptosporidiosis in the UK**

Most human cases of cryptosporidiosis in the UK are due to *C. parvum* or *C. hominis* with other species appearing only occasionally (Chalmers et al. 2009a; Davies & Chalmers, 2009; Elwin et al. 2011). Worldwide, this is the only reported human outbreak of cryptosporidiosis caused by a *Cryptosporidium* species other than *C. parvum* or *C. hominis* (Elwin et al. 2011). Prior to this incident, only one case of *C. cuniculus* infecting a human had been reported (Robinson et al. 2008).
However, it is now clear that *C. cuniculus* is a human pathogen (Chalmers et al. 2009b). Subsequent investigations have identified further sporadic cases in the UK (Chalmers et al. 2014).

**Monitoring for Cryptosporidium oocyst contamination in potable water supplies**

Although not statutorily required to, the water company involved in this incident routinely checked for *Cryptosporidium* oocysts in both the raw and treated parts of the treatment system with continuous filtration cartridges placed at strategic points in the water flow. These cartridges were periodically changed and examined for oocysts. Although filtration analysis can detect contamination, it cannot determine viability, species type or pathogenic potential. For these reasons and because local immunity contributes to whether there is a hazard posed by the organism, there is no specific UK regulation standard for acceptable counts of oocyst contamination of potable water supplies. However, drinking water must not contain parasites at a concentration that could affect human health. Water companies adopt a risk management approach for controlling water supply pathogens informed by the World Health Organization Water Safety Plan for drinking water standards (World Health Organization 2011).

**Demographic characteristics of this outbreak**

**Sex ratio**

The predominance of females affected in this incident is unusual, although other outbreaks have shown similar patterns (MacKenzie et al. 1994; Mason et al. 2010). The difference approaches significance. Possible explanations include: first, although men drink more liquid per day than women (East 2005), women consume more unboiled tap water as a proportion of their intake possibly increasing their exposure. The water consumption data of cases do not support this; however, it is important to consider the possibility of inaccuracies as a result of recall bias, inaccuracy in consumption estimates and assumptions made in the analysis where quantities were not clearly given (e.g. one cup). Secondly, men are less likely to seek medical advice so that positive microbiology and formal notification may have been less available from them (Galdas et al. 2005; Noone & Stephens 2008). Thirdly, there may be a behavioural explanation, such as timing of consumption of plain water in males vs females. Finally, there may be an unexplained difference in response to infection between the sexes. The outbreak was caused by *C. cuniculus* gp60 gene subtype family Va (Chalmers et al. 2009c). In subsequent investigations, it has been found that in sporadic *C. cuniculus* cases the proportion of females affected is greater than males with Va subtype than Vb (Chalmers et al. 2011).

**Children and observed age pattern**

Sporadic cryptosporidiosis mainly affects children aged 1 to 5 years in the UK. Even in waterborne outbreaks, where there is often an increase in adult cases, children are mainly affected (Davies & Chalmers 2009). For example, in the outbreak of *C. parvum* in Clitheroe, Lancashire, UK, 52% of cases occurred in children <5 years old (Howe et al. 2002).

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**Table 1: Clinical characteristics of cases of Cryptosporidium cuniculus**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. (%) of all cases reporting</th>
<th>Significance of difference between sexes (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watery diarrhoea</td>
<td>22 (100)</td>
<td>–</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (18)</td>
<td>0.91</td>
</tr>
<tr>
<td>Nausea</td>
<td>14 (64)</td>
<td>0.86</td>
</tr>
<tr>
<td>Mucousy diarrhoea</td>
<td>5 (23)</td>
<td>0.68</td>
</tr>
<tr>
<td>Abdominal pain&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17 (77)</td>
<td>0.68</td>
</tr>
<tr>
<td>Abdominal cramps&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16 (73)</td>
<td>0.14</td>
</tr>
<tr>
<td>Flushes</td>
<td>10 (48)</td>
<td>0.27</td>
</tr>
<tr>
<td>Fever</td>
<td>10 (46)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Potential clinical risk factor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any history of bowel problems</td>
<td>10 (46)</td>
<td>0.22</td>
</tr>
<tr>
<td>Any other medical history including diabetes, rheumatological, immune suppression, prior radio or chemotherapy (none current)</td>
<td>11 (50)</td>
<td>0.34</td>
</tr>
<tr>
<td>Acid suppression medication/antacids</td>
<td>6 (27)</td>
<td>0.49</td>
</tr>
<tr>
<td>On other medication of any sort (including oral contraceptive but excluding acid suppressors)</td>
<td>10 (46)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

<sup>a</sup>Patient selected.

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This outbreak was unusual as no cases in children under 5 years old were microbiologically confirmed, reported or epidemiologically linked. Plausible explanations include:

- the volume of water consumed by young children was insufficient to provide an infectious dose; the alert occurred early in the morning, giving time for parents to protect their children with alternative drinking water sources or adults avoided giving potentially contaminated water to their children, but took less care for themselves. It is unlikely these potential explanations would hold for all children so some cases in this age group would have been expected. It should be noted that the HPA’s syndromic surveillance system (S. Smith, Health Protection Agency, West Midlands, 2011 personal communication) showed an increase in diarrhoea reports at the time in children in this age group from the affected area, suggesting an ascertainment bias in the local reporting of cases.

Recent evidence on unusual cases of cryptosporidiosis indicates that the median age is older in non C. parvum/ C. hominis infection in the UK (Chalmers et al. 2011; Elwin et al. 2011). Infection due to C. cuniculus matches this pattern. For some unusual Cryptosporidium spp., this may be due to differential exposure, e.g. foreign travel (however, foreign travel as an explanation does not apply to this outbreak).

**C. cuniculus infection characteristics, surveillance and risk factors**

**Incubation period**

The incubation period estimated for this incident may be under or overestimated: the continuous filtering sample methodology does not allow precise estimation of when oocysts first contaminated the final water. Contamination could have occurred at any stage in that 4-day period, although the 165-fold increase in count obtained from the sample taken between 23 and 24 June makes it reasonable to assume that the major contamination occurred on 23 June. The oocyst release was unlikely to be a single ‘pulse’ event; some lower level contamination may have occurred earlier which might explain the otherwise apparently short incubation period experienced by the first case (symptoms commenced on 24 June). The date of contamination at the treatment works may not have been the date of exposure as the transit time of water through the distribution system is not uniform nor would water consumption behaviour be the same in all cases. A single exposure date to calculate the incubation period is therefore artificial. The peak oocyst counts were noted in the network samples between 25 and 27 June with the mean onset date 2 July. It is possible that the incubation period was therefore closer to 7 rather than the calculated mean of 9.2 days. However, in the absence of more precise data, other incubation period approximations would be speculative.

**Surveillance and attack rate**

It is surprising that few confirmed cases were identified given that approximately 258,000 people were potentially exposed (Drinking Water Inspectorate 2009). It is likely that the number of cases identified through the active surveillance implemented following the incident is an underestimate. There is evidence to support this. A report for the Consumer Council for Water interviewed individuals affected by the incident and noted that while some had been ill, none had sought medical attention (Hunt et al. 2008). A study examining syndromic data (NHS Direct data and GP consultations – Q Surveillance) identified a 25% excess above baseline of diarrhoea cases from the area at the time of the incident (Smith et al. 2010) with an absolute excess of 422 cases above normal. This is compatible with an established estimate of 15:1 for the true burden of disease for C. parvum and C. hominis compared with the number of confirmed cases (Nichols et al. 2006; Smith et al. 2010). Other research has suggested a lower ratio of 8.2 community cases to those notified and recorded in national surveillance data (Tam et al. 2012). Nonetheless, the potential for differences in case ascertainment between the increased surveillance implemented as a result of the incident and that for routinely collected surveillance data (used to produce the above ratios) may make such comparisons invalid. However, if correct, the disparity observed between notified and estimated excess cases may suggest that C. cuniculus has comparable levels of population level impact to C. parvum and C. hominis and should therefore be viewed as a significant cause of waterborne disease.
Volume of water

The 2008 Phase-Two (summer) National Tap Water Survey (NTWS) reports a mean of 1.329 L of tap water or 2.003 L total fluid consumption per day – including other sources (East 2008). The mean mains (tap) water consumption in cases arising from the incident was 2.4 L and median 2.3 L (IQR 1.6–3.3). The distribution of the consumption data was not normal and could not be normalised. Formal statistical comparison of the mean water consumption from the outbreak to that reported in the NTWS report is therefore not possible. Nonetheless, the outbreak consumption data possibly suggests a greater level of intake than is typically seen nationally. Greater dosing via higher water volumes consumed may provide an explanation for why these individuals were affected.

Future NTWS reports could usefully describe all measures of central tendency as well as the mean for water consumption to allow comparison to intake noted in outbreaks (as in outbreak situations, small datasets are unlikely to be normally distributed).

Distance from the water treatment works

The duration of diarrhoea was statistically significantly negatively correlated to the direct distance from the water treatment works. This may be a chance finding; however, it could be that oocyst counts were lower at points further from the treatment works and viability could have also declined over distance. However, there was evidence of some concentration of oocysts in storage reservoirs and at customer taps but these were variable with some very low counts taken at similar times as higher counts at other locations. The complex structure of the network produces variable flows of water over time and therefore unpredictable pathogen distribution. Early in the incident (25 June), only four end-user points were tested. These may not have been representative of oocyst load elsewhere at the same time when the loading was possibly at its highest. Nonetheless the magnitude of C. parvum infective dose influences the time to and duration of oocyst excretion, but not clinical incubation period or severity of illness (DuPont et al. 1995). However, others have found longer incubation periods with lower infective doses (Chalmers & Davies 2010). It is possible that diarrhoea duration is consistent with oocyst excretion duration and therefore the infective dose ingested might explain this finding.

Infective dose

The volume of water in the network would have diluted the number of oocysts in any single litre of water. This supports the generally accepted view that the number of organisms required to be ingested to cause symptomatic infection is very small (DuPont et al. 1995; Chalmers & Davies, 2010). The maximum concentration of oocysts per litre of water at the treatment works was below the former regulation treatment standard of <1 oocyst per 10 L of water (ceased 22 December 2007) (Drinking Water Inspectorate 2009), indicating the potential for infection to occur with very low counts of C. cuniculus, as has been demonstrated in previous C. parvum and C. hominis outbreaks (Mason et al. 2010).

Control measures and hazards

Boil water notice

A boil water notice was instituted early on 25 June. A risk/benefit-based decision to remove the boil water notice was made on 4 July. Four cases occurred after this date, two the day after the notice was lifted who would have probably been incubating the infection already and the others occurring on 13 and 14 July respectively, who were thought to be secondary cases and therefore unlikely to have acquired the infection from consuming unboiled tap water. The boil water notice removal appears appropriate despite sporadic oocyst detections from the network beyond that date (assuming the secondary cases were not independently infected from the very low residual counts rather than from an infected contact and that routine surveillance systems did not miss other cases). Continued sporadic oocyst detections in the water network pose a dilemma for decision makers over what constitutes an acceptable count to allow for lifting a boil water notice. It cannot be determined from this incident whether similar residual contamination parameters from a future C. hominis or
The C. parvum incident could be used to determine when to lift a boil water notice as the differing species may have differing pathogenicity and infectivity patterns.

**Hazard**

This outbreak has demonstrated the hazard posed by wildlife to the safety of mains potable water supplies. The Drinking Water Inspectorate, although supportive of the water company’s handling of the incident, was critical of their maintenance arrangements (Drinking Water Inspectorate 2008, 2009). The importance of oocyst-typing to aid source identification was highlighted. Although the source was found early, had this not been the case, timely knowledge of the Cryptosporidium species or genotype could have been helpful for directing investigations and control measures (Drinking Water Inspectorate 2008).

**CONCLUSIONS**

This outbreak was classified as being strongly associated with the consumption of mains drinking water on the basis that the pathogen identified in clinical cases was also found in water samples from the treatment works (Tillett et al. 1998).

C. cuniculus has conclusively been demonstrated to be a human pathogen (Chalmers et al. 2009b). The constellation of symptoms is similar to, but with some differences to other Cryptosporidium spp., especially the age and sex profile, as shown in Table 2 (Chalmers et al. 2009a). Recent work investigating the epidemiology of sporadic C. cuniculus infection has corroborated these findings (Chalmers et al. 2011). It is not possible to conclude from this outbreak whether the observed epidemiological characteristics of C. cuniculus are unique to this species or artifactual. However, other ‘unusual’ Cryptosporidium sp. differ in their epidemiology from C. parvum and C. hominis, although the numbers of cases are small and therefore conclusive differences are difficult to currently ascertain (Elwin et al. 2011).

**Table 2** Epidemiological comparison between this outbreak of Cryptosporidium cuniculus and an outbreak of Cryptosporidium parvum in Clitheroe, Lancashire, UK

<table>
<thead>
<tr>
<th>Epidemiological feature</th>
<th>Percentage of all cases reporting from this incident</th>
<th>Clitheroe outbreak (Cryptosporidium parvum) (Howe et al. 2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age below 5 years</td>
<td>0</td>
<td>52</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>30/70</td>
<td>52/48</td>
</tr>
<tr>
<td>Vomiting</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>77</td>
<td>83</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>73</td>
<td>–</td>
</tr>
<tr>
<td>Fever</td>
<td>45</td>
<td>51</td>
</tr>
</tbody>
</table>

**ACKNOWLEDGEMENTS**

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Richard L. Puleston, Cathy M. Mallaghan, Jonathan S. Nguyen-Van-Tam, Christopher M. Regan are now all affiliated to Public Health England (PHE) following the abolition of the Health Protection Agency and absorption of its functions into PHE. Deborah E. Modha is currently affiliated to University Hospitals Leicester. Gordon L. Nichols is now affiliated to European Centre for Disease Prevention and Control, Stockholm, 17183 Stockholm, Sweden.

**CONFLICT OF INTEREST STATEMENT**

Richard L. Puleston was previously a domestic water customer of Anglian Water. Cathy M. Mallaghan and Deborah E. Modha are domestic water customers of Anglian Water. Paul R. Hunter was until 2010 chair of the science advisory board of Suez Environment and has acted as an expert medical witness in legal cases relating to waterborne outbreaks. Jonathan S. Nguyen-Van-Tam is a domestic water customer of Anglian Water.
customer of Anglian Water. The other authors have no conflicts of interest to declare.

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